

REMARKS

The Examiner is thanked for the withdrawal of the rejection under 35 U.S.C. § 103 over Cantor U.S. Patent No. 5,633,003 ("Cantor") in view of Green WO 96/19968 ("Green").

Obviousness Rejection

Claims 31-33 and 37-47 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Cantor in view of Sanders et al., S.T.P. Pharma Sciences (1997), 7(4), 300-306 ("Sanders"). (Paper 20090610 at 2.)

Cantor has been summarized previously on the record.

The Examiner provided only an Abstract of Sanders, although the article is written in the English language. The complete text of Sanders is submitted, attached, as Exhibit A.¹

Sanders discloses a comparative study of "the pulmonary deposition of ultrafine and fine particle size aerosols generated by different formulations in healthy volunteers and in asthmatic subjects. The two formulations used were a Teflon particulate suspension with a mean particle diameter of 5.76 μm , and a solution of radiolabel in propellant which generated aerosol particles of 2.13 μm mean diameter. A standard metered dose inhaler formulation [using chlorofluorocarbon propellants] was utilized for both aerosols. Both formulations were radiolabelled with Tc-99m [and]

¹ Sanders is also listed in an accompanying Supplemental Information Disclosure Statement.

[p]ulmonary deposition of the aerosols was measured... The aerosols generated from the solution produced a significantly greater total lung deposition compared to the suspension aerosol in both asthmatic and healthy subjects..." (Abstract, lines 1-15.)

In making the rejection, the Examiner asserted that "Cantor discloses a system for delivering a glycosaminoglycan or polysaccharide formulation to a respiratory tract of a mammal, comprising: a mixture comprising a polysaccharide or glycosaminoglycan (hyaluronic acid), that can be delivered via a route aerosol inhalation by a nebulizer (see col. 3, METHODS, lines 46 to col. 4, line 45; also, see abstract). In addition, Cantor uses the same method of delivery (aerosol inhalation) for the same purpose (i.e., treating respiratory disorders) comprising a glycosaminoglycan or polysaccharide. Furthermore, it should be noted that the nebulizer contains the said canister, valve and nozzle, claimed by applicant. Also, Cantor discloses that the hyaluronic acid used may be derived from bovine sources, rooster comb, human umbilical cord, or streptococcus zoepidicus (see col. 3, lines 13-18). This implies that hyaluronic acid of different molecular weights can be used since the said sources of hyaluronic acid produces hyaluronic acid of different molecular weight. In fact, the hyaluronic acid suggested by Cantor are naturally occurring hyaluronic acid (i.e., hyaluronic acid from bovine sources, rooster comb, human umbilical cord, or streptococcus zoepidicus (see col. 3, lines 13-18) which are known to have molecular weight of 50,000-13,000,000 daltons (for example, see US 4,746,504: col. 4, lines 44-49)." (Paper 20090610 at 3-4.) The Examiner also asserted that "this molecular weight range of hyaluronic acid encompasses the molecular weight range of the hyaluronic acid claimed..." (Id. at 4.)

The Examiner further asserted that “[t]he difference between applicants’ claimed composition and the composition of Cantor is that Cantor does not disclose the concentration, molecular weight of the polysaccharide or glycosaminoglycan and Cantor does not use a drug or propellant. However, Cantor suggests that hyaluronic acid from different sources (i.e., hyaluronic acid from bovine sources, rooster comb, human umbilical cord, or streptococcus zoepidicus (see col. 3, lines 13-18)) which are known to have different molecular weights can be used and Cantor disclose that the effective daily amount of hyaluronic acid is from about 10 µg/kg to about 1 mg/kg of body weight (see abstract and col. 2, lines 47-67). This suggest that the concentration can encompass the concentration claimed by applicant since the mass and thus the concentration to be prepared depends on the body weight (kg) of the recipient and especially since Cantor exemplifies a concentration (1.0 mg/0.2ml or 5.0 mg/ml) that is substantially close to applicant’s claimed concentration.” (Id. at 4-5.)

Furthermore, the Examiner asserted that “Sanders et al. disclose that propellant-soluble drugs delivered from metered dose inhalers may offer better airway penetration in both normal and asthmatic subjects (see abstract). Sanders et al. uses a chlorofluorocarbon propellant as propellant (see abstract).” (Id. at 5.)

The Examiner concluded that “[i]t would have been obvious to one having ordinary skill in the art, at the time the claimed invention was made to have prepared the composition (an inhalant aerosol formulation) of Cantor comprising different concentrations, molecular weights or particle size of the polysaccharide or glycosaminoglycan with a fluorocarbon propellant to be used as an inhalant aerosol formulation for treating respiratory conditions or disorders, depending on factors such as

the severity of the respiratory disorder or the type, age and weight of subject treated, since Cantor suggests that different molecular weights of hyaluronic acid (polysaccharide) can be used and Sanders et al. disclose that propellant-soluble drugs delivered from metered dose inhalers may offer better airway penetration in both normal and asthmatic subjects.” (Id. at 5.)

The Examiner also concluded that “[o]ne having ordinary skill in the art would have been motivated, to prepare the composition (an inhalant aerosol formulation) of Cantor comprising different concentrations, molecular weights or particle size of the polysaccharide or glycosaminoglycan with a fluorocarbon propellant to be used as an inhalant aerosol formulation for treating respiratory conditions or disorders, depending on factors such as the severity of the respiratory disorder or the type, age and weight of subject treated, since Cantor suggests that different molecular weights of hyaluronic acid (polysaccharide) can be used and Sanders et al. disclose that propellant-soluble drugs delivered from metered dose inhalers may offer better airway penetration in both normal and asthmatic subjects.” (Id. at 5-6.) The Examiner also asserted that “it is obvious to combine other drugs such as terbutaline and Beclomethasone (which are often used for asthma treatment) with the hyaluronic acid or glycosaminoglycan since they have the same utility.” (Id. at 6.) The Examiner further asserted that “it is obvious to use other polysaccharides including polysaccharides that are conjugated to a drug since both Cantor disclose [sic] the use of polysaccharides in general.” (Id.)

It is well settled the Examiner bears the burden to set forth a *prima facie* case of unpatentability. *In re Glaug*, 62 USPQ2d 1151, 1152 (Fed. Cir. 2002); *In re*

Oetiker, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992); and *In re Piasecki*, 223 USPQ 785, 788 (Fed. Cir. 1984). If the PTO fails to meet its burden, then the applicant is entitled to a patent. *In re Glaug*, 62 USPQ2d at 1152.

Beyond looking at the cited documents to determine if any of them suggests doing what the inventors have done, one must also consider if the art provides the required expectation of succeeding in that endeavor. See *In re Dow Chem. Co. v. American Cyanamid Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988) ("Both the suggestion and the expectation of success must be founded in the prior art, not in applicants' disclosure.") "Obviousness does not require absolute predictability, but a reasonable expectation of success is necessary." *In re Clinton*, 188 USPQ 365, 367 (CCPA 1976). Furthermore, the U.S. Patent and Trademark Office Examination Guidelines at page 57527 provide the following guidance to Examiners: "In short, the focus when making a determination of obviousness should be on what a person of ordinary skill in the pertinent art would have known at the time of the invention, and on what such a person would have reasonably expected to have been able to do in view of that knowledge". However, no such motivation or expectation of success can be found in the cited documents.

Arguments submitted on the record are incorporated herein.

Known options for formulation and delivery of an agent in various combinations targeting the lungs would not have provided predictable results. Numerous formulation and delivery parameters would have been available to one skilled in the art. Formulation for aerosol delivery is also complicated by known difficulties such as loss of the delivered agent during inhalation, dosing difficulties,

enzymatic degradation in the lung, the complex anatomical structure of the respiratory system of a mammal including structures divided into many folds, non-uniform distribution of agents, varying effects of different molecular weight particles within the lungs as well as delivery of a desired molecular weight agent in relation to that administered, particle size distribution, etc.

Furthermore, one skilled in the art would not have expected success in achieving the claimed system for pulmonary delivery to a mammal of an inhalable mist of a glycosaminoglycan in an amount effective to coat elastic fibers of the lung to protect the fibers from injury by an elastase, based on Cantor alone or in combination with Sanders. Developing a system in which delivery of a glycosaminoglycan is in an amount effective to coat elastic fibers of the lung to protect the fibers from injury by an elastase is further complicated in that coating to protect the fibers involves achieving a sufficient coating of fibers on the lung surfaces, rather than aerosol delivery of an agent for absorption by lung tissue, where the agent is, for example, a drug or in some cases a polypeptide.

One skilled in the art would not have been able to predict how the claimed molecular weight range parameter of a glycosaminoglycan would affect the droplet size and viscosity of the aerosolized material as well as penetration into the airways and coating of the fibers. The Examiner's mere citation of naturally available molecular weight ranges of glycosaminoglycans of "50,000-13,000,000 daltons" (Paper No. 20090610), does not address the considerations that would have faced one skilled in the art in developing a system for pulmonary delivery, as claimed. With the goal of protecting the extracellular matrix against damage, one skilled in the art would have

considered a high molecular weight preparation desirable "in order to provide effective binding to and coating of elastin fibers." (Specification, page 19, lines 27-30; see generally page 19, line 17-page 21, line 27.) In particular, the specification discloses that "[i]t has been observed that higher molecular weight preparations of polysaccharides: (1) persist longer in the lungs, (2) hold more water, (3) provide greater supplementation of elastic recoil, and (4) provide thicker and more complete coverage of extracellular matrix, than lower molecular weight preparations." (Page 20, lines 15-18.) Yet applicants have discovered, as is disclosed in the specification, that "[w]hen a preparation of HA having a molecular weight of greater than 2,000,000 Daltons was used, it produced a solution that was excessively viscous." (Page 20, lines 28-30) (emphasis added.) In short, the rejection is deficient at least because it fails to identify any disclosure or suggestion of a nexus between the identification of various natural sources of HA (Cantor) and use of specific molecular weight ranges of glycosaminoglycans to protect lung fibers against injury by an elastase in a system for pulmonary delivery as presently claimed.

Indded, the claimed system recites a molecular weight at the lower end of the range cited by the Examiner, namely in which a glycosaminoglycan has a molecular weight of between about 50,000 and 1.5×10^6 Daltons. One skilled in the art would not have predicted that the claimed molecular weight range would have been suitable, and furthermore, one would have been led away from the claimed molecular weight range because he or she would have considered the higher molecular weight preparation as suitable to potentially coat lung fibers. In addition, had one skilled in the art, other than applicants, found that molecular weights higher than 2,000,000 daltons produce a

solution that would be too viscous for use in a system for pulmonary delivery to a mammal of an inhalable mist of a glycosaminoglycan, this would further have deterred one from attempting to achieve the goals of the claimed system.

And, one skilled in the art would not have been able to predict which concentration would be effective, in combination with the recited molecular weight, for use in the claimed system, for example, to aerosolize and deliver a useful amount for coating the fibers. As the specification discloses, "[b]esides the molecular weight, the concentration of the glycosaminoglycan solution also influences duration times, water retention, elastic recoil, and matrix coverage, and formulation viscosity. (Page 21, lines 6-8; see also lines 7-20.) Yet the selected concentration, in conjunction with the recited molecular weight range of HA, was found to be important in achieving suitable droplet size distributions. (Specification, Example 23, particularly page 61, lines 2-4.)

In particular, one skilled in the art could not glean any predictive information from Cantor regarding whether or not any particular molecular weight and concentration parameter (and/or any other parameters) in combination would achieve the desired goals of the claimed system. Cantor's disclosure of an example of a 1.0 mg/0.2 ml concentration administered (Cantor, Col. 4, lines 25-29), as noted by the Examiner (Paper No. 20090610 at 5), is of intratracheal administration. As acknowledged by the Examiner, "Cantor does not use a ... propellant." (Id. at 4.) Many of the factors involved in formulating for intratracheal delivery as exemplified in Cantor differ from and do not inform as to the parameters for delivery of an inhalable aerosol mist using a breathable fluorocarbon propellant. Factors involved in delivery using a fluorocarbon propellant include, as would be known to one of skill in the art, for

example, the amount deposited in the mouth and throat prior to reaching the lungs and the amount that may be exhaled before it is able to be deposited on the elastic fibers, the effect of the propellant on parameters such as droplet size, etc. See the specification at, for example, page 22, line 14 to page 25, line 25. Any effects of a disclosed concentration and/or other parameters involving the use of a glycosaminoglycan in intratracheal administration as disclosed by Cantor cannot be extrapolated, as would be known to one of skill in the art, to predict effects in a hypothetical system as claimed which uses a propellant, for pulmonary delivery to a mammal to coat elastic fibers of the lung to protect the fibers from injury by an elastase.

Furthermore, the Cantor example involves not only a different manner of delivery, intratracheal, but also that the HA was administered "immediately" "[f]ollowing intratracheal administration of 20 units of pancreatic elastase." (Col. 4, lines 25-29.) The administration by Cantor of HA using the cited concentration is affected not only by the different mode of delivery, i.e., intratracheal, but also by the effects of administering the additional and particular agent, pancreatic elastase, as well as the relative order of administration. Because the claimed system involves protecting the fibers of the lung from injury by an elastase, one skilled in the art would be led away from parameters of the claimed system in view of Cantor's disclosure of the administration of pancreatic elastase immediately prior to HA, which would be counter to the recited purpose in the preamble and thus, the desired results of the claimed system.

As noted above, known options for formulation and delivery of a glycosaminoglycan in various combinations would not have provided predictable results in achieving a system for pulmonary delivery to a mammal of an inhalable aerosol mist

of a glycosaminoglycan in an amount effective to coat elastic fibers of the lung to protect the fibers from injury by an elastase, which uses a fluorocarbon propellant. Yet the system as presently claimed provides a more uniform distribution of HA to elastic fibers than that seen with intratracheal installation which resulted in "patchy" distribution. (Specification, Examples 5 and 6.) Furthermore, aerosolized HA in hamsters was shown to have a protective effect against neutrophil elastase, which is involved in the pathogenesis of human emphysema; *in vitro* data also supports this conclusion. (Specification, Examples 5-15.)

Reliance on Sanders to fill the gaps in the Examiner's citation of Cantor is unfounded. Sanders discloses a study comparing "different formulations" (Abstract, line 3), not only with regard to the respective particle size, but notably also the substances delivered differ and how they are formulated differ. Sanders discloses that the two formulations used were a labeled Teflon particulate suspension with a mean particle diameter of 5.76 μm , namely technetium-99m labeled Teflon, and a solution of radiolabel in propellant which generated aerosol particles of 2.13 μm mean diameter, namely the technetium-99m-hexakis-(t-butylisonitrile) complex. (Abstract, lines 4-7; page 301, right column, first 4 lines under section I.2; page 302, left column, first 4 lines under section I.3.) Sanders also points out that "there is a significant difference in the nature of the inhaled droplets [in terms of their respective compositions], in that the suspension aerosols consist of solid polymer particles, while the major component of the solution system is the surfactant, which will form an oily semisolid mass once the propellant has evaporated." (Page 305, left column, lines 44-48.) Sanders acknowledges that "it is possible that this may create differences in the deposition of the

two formulations; in this regard, it should be noted that the effect of particle composition has been poorly investigated..." (Page 305, left column, lines 48-51) (emphasis added.)

Moreover, Sanders does not deliver to the lungs a drug or any drug-like compound. One skilled in the art would consider that the labeled Teflon particles and radiolabel tested in Sanders, again different substances compared, would have little or no predictive value with regard to aerosol delivery of a drug, nonetheless a glycosaminoglycan. For all of the above reasons, the Examiner's assertion that "Sanders et al. disclose that propellant-soluble drugs delivered from metered dose inhalers may offer better airway penetration in both normal and asthmatic subjects" is quite speculative, at best for the Examiner.

Furthermore, it would be apparent to one of skill in the art that a study on the deposition of labeled Teflon particles and radiolabel in the lung would not be informative in attempting to develop a system for pulmonary delivery to a mammal of an inhalable aerosol mist of a glycosaminoglycan in an amount effective to coat elastic fibers of the lung to protect the fibers from injury by an elastase. First, it is apparent that labeled Teflon particles and the indicated radiolabel differ markedly from glycosaminoglycan molecules. Teflon is a polymer, such as the fluorinated ethylene propylene used in Sanders. (Page 301, right column, first para after section 2.) As is well known, Teflon is used in film coatings, bakeware, and in other industrial applications. The radiolabel used by Sanders is known as a cardiac imaging agent. One skilled in the art would understand that neither Teflon particles nor the radiolabel would act in the lung in a manner that could predict any aspect of the development of a system for pulmonary delivery to a mammal of an inhalable aerosol mist of a

glycosaminoglycan in an amount effective to coat elastic fibers of the lung to protect the fibers from injury by an elastase, as claimed.

Unlike Teflon particles and the radiolabel which are foreign materials in a mammalian organism, glycosaminoglycans are endogenously found. (Cantor, Col. 3, lines 13-16.) And, they are relatively large molecular weight polysaccharides. Glycosaminoglycans such as HA retain water (see the specification, e.g., at 21, lines 6-8) and have charge interactions that differ from each of the Teflon particles and the radiolabel. The specification indicates that ability of HA to retain water and its particular charged carboxyl groups attached to the saccharide moieties of HA may be involved in the ability of HA to limit injury to elastic fibers. (Page 10, lines 22-27.) Also, Sanders focuses on particle size and makes no mention of molecular weight as an important parameter. More importantly, the compositions tested in Sanders consist of different substances, as noted above, and it is not clear what respective molecular weights are involved as between the Teflon particles and the radiolabel. The specification discloses, however, that in the claimed invention, "a molecular weight ... is selected to produce a desired physiologic effect or molecular interaction, i.e., a desired therapeutic profile." (Page 19, lines 17-19.)

There is no indication that the use of Teflon particles or the radiolabel by Sanders in any way provides information regarding parameters for delivery of a glycosaminoglycan such as the recited molecular weight range and concentration of the claimed system. Nor does Sanders provide any notion as to achieving success in the claimed system. One skilled in the art simply would not consider Teflon particle or radiolabel deposition in the lungs to be informative regarding the claimed system for

pulmonary delivery to a mammal of an inhalable aerosol mist of a glycosaminoglycan in an amount effective to coat elastic fibers of the lung to protect the fibers from injury by an elastase, as claimed. This is underscored by the fact that it is well known that Teflon is an irritant in the lungs.²

Known options for combination, in this case for delivery of a glycosaminoglycan for pulmonary delivery, were not “finite, identified, and predictable”, as in the facts presented in *KSR Int. Co. v. Teleflex, Inc.*, 127 S. Ct. 1727 (2007). In *Abbott Labs. v. Sandoz, Inc.*, 89 USPQ 1161, 1171 (Fed. Cir. 2008), the Court of Appeals for the Federal Circuit indicated that the Supreme Court in *KSR* “did not create a presumption that all experimentation in fields where there is already a background of useful knowledge is “obvious to try,” without considering the nature of the science or technology.” Indeed, the Federal Circuit has recently reiterated that “merely [throwing] metaphorical darts at a board filled with combinatorial prior art possibilities” is the epitome of impermissible hindsight reconstruction. *In re Kubin*, slip op. 2008-1184, 14 (Fed. Cir. April 3, 2009).

As in the *Abbott* case involving the problem of producing extended release formulations having recited pharmacokinetic properties, one skilled in the art would not

² Enclosed as Exhibit B is the Abstract of Hardie, W. D., et al., “Attenuation of acute lung injury in transgenic mice expressing human transforming growth factor- α ”, *Am. J. Physiol. Lung Cell Mol. Physiol.* 277: L1045-L1050, 1999. The Abstract discloses that in order to test the effect of human transforming growth factor- α , “TGF- α transgenic and nontransgenic control mice were exposed to polytetrafluoroethylene (PTFE; Teflon) fumes **to induce acute lung injury.**” (Abstract, lines 7-10) (emphasis added.) Thus, at least one of the agents delivered by Sanders is harmful for delivery *in vivo* to the lung.

have anticipated success in achieving the presently claimed system, as "knowledge of the goal does not render its achievement obvious." *Abbott Labs. v. Sandoz, Inc.*, 89 USPQ at 1172 (affirming the district court's determination that Abbott is likely to prevail in its claim that the patent is valid, and upholding the grant of a preliminary injunction). We respectfully submit that the rejection has done no more than launch "metaphorical darts" based on the present disclosure where numerous formulation, delivery, and composition options would have been known but a result not predictable, and for this reason alone the rejection must be withdrawn.

It is also submitted that even if a combination of Cantor and Sanders were proper, which we submit it is not, the combination would not result in the claimed system.

It is submitted that the rejection has been rendered moot. Reconsideration and withdrawal of the rejection are requested.

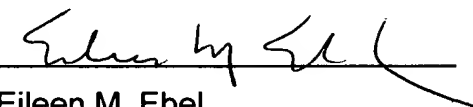
In view of the foregoing, withdrawal of the outstanding rejection is respectfully requested. It is submitted that the application is in condition for allowance. Issuance of a Notice of Allowance is respectfully requested.

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on October 23, 2009.



Eileen M. Ebel, Reg. No. 37,316

Respectfully submitted,

By: 

Eileen M. Ebel
Registration No. 37,316
BRYAN CAVE LLP
1290 Avenue of the Americas
33rd Floor
New York, NY 10104-3300
Phone: (212) 541-2000
Fax: (212) 541-4630

The deposition of solution-based and suspension-based aerosols from metered dose inhalers in healthy subjects and asthmatic patients

P. Sanders¹, N. Washington^{2*}, M. Frier³, C.G. Wilson⁴, L.C. Feely⁵ and C. Washington⁶

¹Department of Physiology and Pharmacology, ²Department of Surgery, and ³Department of Medical Physics, Medical School, Queens Medical Centre, Nottingham NG7 2UH, United Kingdom

⁴Department of Pharmaceutical Sciences, Royal College, University of Strathclyde, Glasgow G1 1XW, United Kingdom

⁵Abbott Laboratories, Ltd., Queenborough, Kent ME11 5EL, United Kingdom

⁶Department of Pharmaceutical Sciences, University Park, University of Nottingham, Nottingham NG7 2RD, United Kingdom

*Correspondence

This study was carried out to compare the pulmonary deposition of ultrafine and fine particle size aerosols generated by different formulations in healthy volunteers and in asthmatic subjects. The two formulations used were a Teflon particulate suspension with a mean particle diameter of 5.76 μm , and a solution of radiolabel in propellant which generated aerosol particles of 2.13 μm mean diameter. A standard metered dose inhaler formulation (vapour pressure 444 kPa, chlorofluorocarbon 11 and 12 propellants, ratio 30/70) was utilized for both aerosols. Both formulations were radiolabelled with Tc-99m. Pulmonary deposition of the aerosols was measured using gamma scintigraphy. The aerosol generated from the solution produced a significantly greater total lung deposition compared to the suspension aerosol in both asthmatic and healthy subjects: $61 \pm 11\%$, compared to $17 \pm 5\%$ for healthy subjects ($p = 0.009$), and $59 \pm 17\%$, compared to $10 \pm 4\%$ in asthmatic subjects ($p = 0.004$). The results suggest that propellant-soluble drugs delivered from metered dose inhalers may offer better airway penetration in both normal and asthmatic subjects.

Cette étude a eu pour objectif de comparer, chez les volontaires sains et chez les sujets asthmatiques, le dépôt pulmonaire de particules d'aérosols fines et ultrafines formées à partir de différentes formules. Les deux formules étudiées étaient une suspension de particules de Téflon d'un diamètre moyen de 5,76 μm , et une solution radiomarquée dans un propulseur générant des particules d'un diamètre moyen de 2,13 μm . Une formule standard pour inhalateur à valve doseuse (pression de vapeur 444 kPa, chlorofluorocarbones 11 et 12, en proportions 30/70) a été utilisée pour les deux types d'aérosol. Les deux formules ont été radiomarquées au Tc-99m. Le dépôt pulmonaire des aérosols a été mesuré par gamma scintigraphie. Les aérosols formés à partir de la solution conduisent à un dépôt pulmonaire total significativement plus important que celui des aérosols sous forme de suspension, aussi bien chez les asthmatiques que chez les volontaires sains: $61 \pm 11\%$ contre $17 \pm 5\%$ chez les volontaires sains ($p = 0,009$) et $59 \pm 17\%$ contre $10 \pm 4\%$ chez les sujets asthmatiques ($p = 0,004$). Ces résultats laissent penser que les principes actifs solubles dans les propulseurs administrés à partir d'inhalateurs à valve doseuse permettent une meilleure pénétration par les voies aériennes chez les sujets sains aussi bien que chez les sujets asthmatiques.

Keywords: Pulmonary deposition — Metered dose inhalers — Asthma — Gamma scintigraphy — Particle size.

Mots clefs: Dépôt pulmonaire — Inhalateurs à valve doseuse — Asthme — Gamma scintigraphie — Taille de particules.

Asthma is described as partial obstruction to air flow in the intrathoracic airways which can vary in severity over short periods of time. Until recently, it was believed that only 5% of the population suffered from asthma at some stage in life, but increasing rates of diagnosis, and possibly aggravation of the disease by poor air quality, have led to a revision of the estimate to as much as 10 to 15%, especially for people in the second decade of life [1].

The majority of patients who suffer from asthma and other obstructive airway diseases are dependent on inhalation therapy for treatment. Metered dose inhalers are the most commonly used devices for this purpose. Generally, these contain fine suspensions of drug particles in chlorofluorocarbon replacements, such as hydrofluoroalkane propellants. The drugs in suspension aerosols are normally ground by fluid energy milling to a diameter of 2 to 5 μm . Metered dose inhalers confer

many advantages over other drug delivery systems: they are cheap, quick to use, easy to carry and multidose. Their primary drawback is that, in healthy people, only approximately 10% of particulates delivered from a single dose of a metered dose inhaler actually reach the lung, since the bulk impacts on the mouthpiece and oropharynx [2].

Delivery to the lung is strongly dependent on particle size. Droplets larger than 10 μm impact in the upper airway, but droplets in the 0.5 to 5 μm range are sufficiently small to penetrate into the lower airways, where they may adhere by impaction. Smaller particles also migrate to the vessel wall by Brownian motion, but any particles which do not deposit in a relatively short time may be lost in the subsequent exhalation. It has been known for some time that the optimum diameter for aerosol penetration in subjects with normal airways is between 2 and 3 μm [3]. In patients with asthma, the airway is narrowed by a combination of bronchospasm, inflammation and mucus secretion, and deposition of drug from an aerosol is more likely to occur in the upper respiratory pathway. The optimal particle size in such patients is, however, still in the region of 3 μm [4], since deep peripheral deposition is not normally the objective in treating such cases.

Previous work carried out in our laboratory studied the pulmonary deposition of the radiolabel technetium-99m hexamethylpropyleneamineoxime. This material is extremely hydrophobic and can thus be prepared as a soluble marker in the propellant chlorofluorocarbon. Administration of this formulation to normal volunteers produced more than 30% total lung deposition in healthy subjects when administered by metered dose inhalers [5, 6]. It was suggested that this was due to the solution generating a particle size on evaporation that was significantly smaller than that which could be easily achieved using suspension aerosols. It would be expected that the particle size generated by evaporation of an aerosol containing an involatile solute would be determined by the total concentration of solutes (drugs, excipients, and surfactants), and could be made very small by using dilute solutions, providing a sufficient dose could be administered. Most drugs are not soluble in chlorofluorocarbons or hydrofluoroalkanes, but many can be made so through the addition of a cosolvent, such as ethanol, or a microemulsion-forming surfactant, such as lecithin [7, 8]. This mechanism presupposes that the propellant is largely evaporated by the time the aerosol reaches the deeper levels of the lungs; we shall develop this point further in due course.

The present study was designed to compare the performance of typical suspension-based and solution-based metered dose inhalers in normal subjects and in patients with asthma. For this purpose, we used monodisperse Teflon particles radiolabelled with technetium-99m pertechnetate, made using a spinning disc generator [9]; these have been well characterized as model suspension aerosols [10-12]. The deposition of these particles was compared to that of an aerosol containing a solution of technetium-99m-hexakis-(*t*-butylisonitrile), a hydrophobic technetium complex which is readily soluble in a range of nonpolar solvents.

1. MATERIALS AND METHODS

1. Metered dose inhalers

Both solution and suspension metered dose inhalers were formulated in conventional 10 ml aluminium canisters with a nitrile O-ring and a 50 μl metering valve. Both formulations contained the chlorofluorocarbons trichlorofluoromethane and dichlorodifluoromethane (Aldrich), which were introduced by cold filling by volume in the ratio of 30/70, respectively, giving a vapour pressure of 444 kPa (64.4 psig) at 25°C. The surfactant sorbitan trioleate (Sigma Chemical Co.) was weighed into the cans directly at a concentration of 1.64 mg/ml. This dissolved easily in the propellant.

2. Suspension-type formulation

Technetium-99m labelled Teflon (fluorinated ethylene propylene, DuPont Ltd., Hertfordshire, United Kingdom) particles were produced with a spinning disc generator [9] using procedures that have been described previously [10-12]. The following procedure was used to optimize the labelling efficiency.

A spinning disc generator was set up on a clean glass plate inside a cylindrical perspex hood behind lead shielding. Isotonic saline (2 ml) containing 100 MBq $^{99\text{m}}\text{Tc}$ sodium pertechnetate was eluted from a molybdenum-99/technetium-99m generator (Amersham, United Kingdom) and the pertechnetate was extracted using butanone (2 x 1 ml) in a separating flask. This extraction procedure was necessary, since it has previously been shown that sodium chloride and hydrochloric acid in the aqueous phase cause subsequent leaching and loss of technetium from the Teflon particles [10, 12]. The organic phase containing the technetium-99m was evaporated to dryness in a stream of nitrogen, and the Teflon suspension (0.5 ml) in 50 ml of 40% ethanol was added to the technetium-99m residue. This solution was run onto the centre of the spinning disc using a syringe pump set at a flow rate of 0.4 ml/min. The spinning disc was driven by compressed nitrogen at a flow rate of 14 l/min, giving a disc speed of 33,000 r/min (checked stroboscopically).

A 300-watt light serving as a heat source was placed outside the perspex hood to enhance the evaporation of the liquid droplets ejected from the disc. The particles generated were allowed to settle onto glass plates on the base of the hood, and any particles which did not settle were captured in a series of traps (3 water, 2 air and 1 fibre). When the preparation was complete, the apparatus was dismantled and the plates were placed in a preheated oven at 240°C for 8 min to sinter the particles. The glass plates were then left to cool and the Teflon particles were collected by scraping with a razor blade; they were weighed directly into a canister containing the surfactant. The canister was then cooled in liquid nitrogen prior to the addition of propellants, and the valve was then crimp-sealed. During this process, precooled equipment was used, and the metered dose inhaler canister was repeatedly chilled in liquid nitrogen to prevent evaporation of the propellants. The canisters

were checked for leaks around the valve crimp by immersion in warm water, and then agitated in an ultrasonic bath for 5 min to disperse the particles. Finally, the valve was primed three to four times and the radioactivity per firing was assayed using a Pitman isotope assay calibrator type 238 ionization chamber and electrometer unit.

3. Solution-type formulation

The trichlorofluoromethane-soluble label technetium-99m-hexakis-(*t*-butylisonitrile) complex was prepared by direct reduction of pertechnetate as described by Angelberger and co-workers [13]. The technetium-99m-hexakis-(*t*-butylisonitrile) complex was extracted from the reaction mixture with chloroform (2 x 1 ml portions), transferred to a metered dose inhaler canister containing the surfactant and evaporated to dryness. The canister was cooled in liquid nitrogen prior to the addition of propellants and then crimp-sealed. Valve crimp leakage, ultrasonic agitation, valve priming and radioactivity assay were performed as described for the Teflon suspension formulation.

4. Aerosol characterization

Size analysis of both aerosol formulations was undertaken using an Andersen eight-stage cascade impactor fitted with a glass throat (Quickfit, RA9/55) on the first stage. Each aerosol was shaken and five actuations were directed into the impactor with an airflow of 28.0 l/min. The impactor was disassembled and the amount of radioactivity on each stage was determined by scintillation counting. The data were expressed as the percentage deposition on each stage of the amount entering the Andersen. From this data, a mass mean aerodynamic diameter was calculated for the metered dose inhaler formulation.

Photographs of the Teflon particles were taken using a scanning electron microscope (figure 1); 148 particle diameters were measured to assess the mean diameter and size distribution. This was a relatively small number, but it did provide adequate statistics due to the relatively low polydispersity of the particles.

5. Study population

Exclusion criteria for all subjects included participation in a clinical trial within the previous three months, consumption of any medication other than the contraceptive pill, tobacco smoking, excessive alcohol intake or pregnancy. The state of health and past medical history of the volunteers was ascertained by medical questionnaire.

5.1. Healthy volunteers

Subjects fulfilling the required entry criteria and having normal respiratory function values of forced vital capacity and forced expiratory volume in 1 s were recruited for the study. Respiratory function measurements were assessed by Vitalograph. Eleven healthy subjects were recruited with lung function parameters as follows: mean age 28.36 years, age range 22 to 58 years, mean forced expiratory volume in 1 s

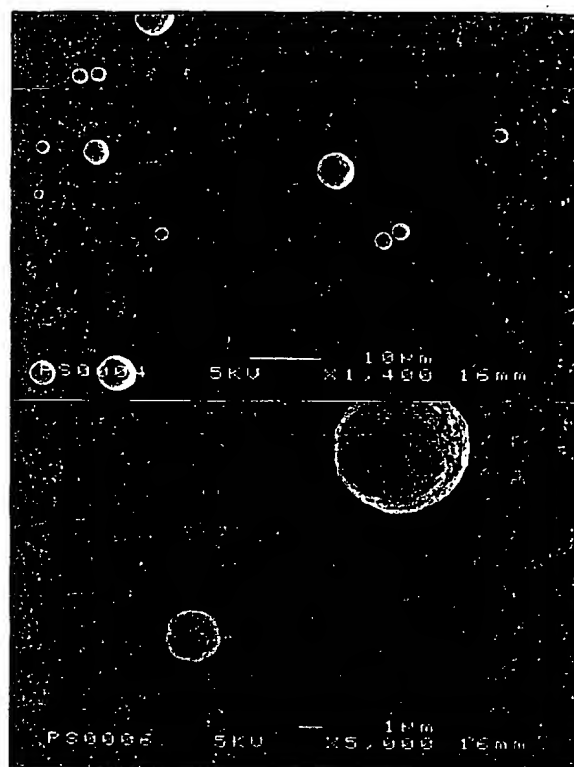


Figure 1 - Electron micrographs of Teflon microparticles at 1400x (upper half) and 5000x (lower half).

4.45 l (range 3.25 to 5.35 l) and forced vital capacity 5.23 l (range 3.90 to 5.75 l). The predicted forced expiratory volume in 1 s was 111% (range 89 to 129%).

5.2. Patients with asthma

Patients with asthma were recruited from the Asthma Register of the Nottingham City Hospital and the Queens Medical Centre NHS Trusts. Patients who entered the study had a history of asthma and were taking regular inhaler medication, but were otherwise healthy. Nine asthma patients were recruited. The mean age was 37.11 years, ranging from 20 to 48 years, the mean forced expiratory volume in 1 s and forced vital capacity were 2.61 l (range 0.79 to 3.95 l) and 3.64 l (range 0.85 to 5.50 l), respectively, and the baseline forced expiratory volume in 1 s was 38 to 123% predicted. Respiratory function measurements were assessed by Vitalograph on each study day a few hours prior to the scintigraphy. The maximum variation showed by any patient among the study days was 12% in forced expiratory volume in 1 s and 15% in forced vital capacity (mean variations 2.7 and 3%, respectively).

6. Ethical considerations

The study was approved by the Nottingham University Medical School and University, and Highbury and General Hospital Ethics Committees. All subjects recruited for the study gave prior written informed consent. The administration

of all the radiopharmaceuticals during the study was approved by the Administration of Radioactive Substances Advisory Committee. The study was performed in accordance with the Declaration of Helsinki (Hong Kong amendment).

7. Administration of radiolabelled aerosol

Subjects were instructed and trained to use a placebo metered dose inhaler correctly by firing it during a period of slow and steady deep breaths, followed by a 10 s breath hold. The subjects were trained until they felt confident with the use of the inhaler and until no signs of bad technique were evident.

Two actuations were inhaled by the subject. The subject was then seated in front of the gamma camera which had been positioned at an appropriate height to obtain an image of the lungs. Anterior and posterior images of the lungs and stomach, and a left lateral head/neck view were acquired. An anterior krypton-81m ventilation image of the lungs was also recorded for each subject during tidal breathing while the subject sat erect in front of the camera [14].

The crossover study was performed after a seven-day washout period. The administration of the formulations was randomized and the subject was blinded to the formulation administered.

8. Analysis of imaging data

A contour line representing the outer margins of the right and left lungs was defined using the anterior krypton-81m ventilation image. This region was used to calculate deposition of the technetium-99m in the lung. Additional regions were drawn around the oesophagus/trachea and stomach, and a region of the background was assessed.

The total number of counts and the total number of cells in each defined region were recorded for each view. Counts were corrected for background activity and radioactive decay. The geometric mean was calculated from the corrected anterior and posterior count rates; this is a well-established procedure to correct for differences in attenuation of the radiation by overlying tissues and variations in anterior/posterior isotope distribution. The counts for each region were then normalized allowing the total lung deposition, as a percentage of the delivered dose, to be calculated.

II. RESULTS

1. Metered dose inhaler sizing

Figures 2a and b show the deposition patterns in the Andersen sampler of the suspension and solution phase aerosols respectively. The suspension-based aerosol had a mass mean aerodynamic diameter of 5.76 μm , and the solution-based aerosol had a mass mean aerodynamic diameter of 2.12 μm . While most of the solution-based aerosol had a mass mean aerodynamic diameter below 5 μm , the suspension-based aerosol had approximately 35% of the total mass above 7 μm . It was not

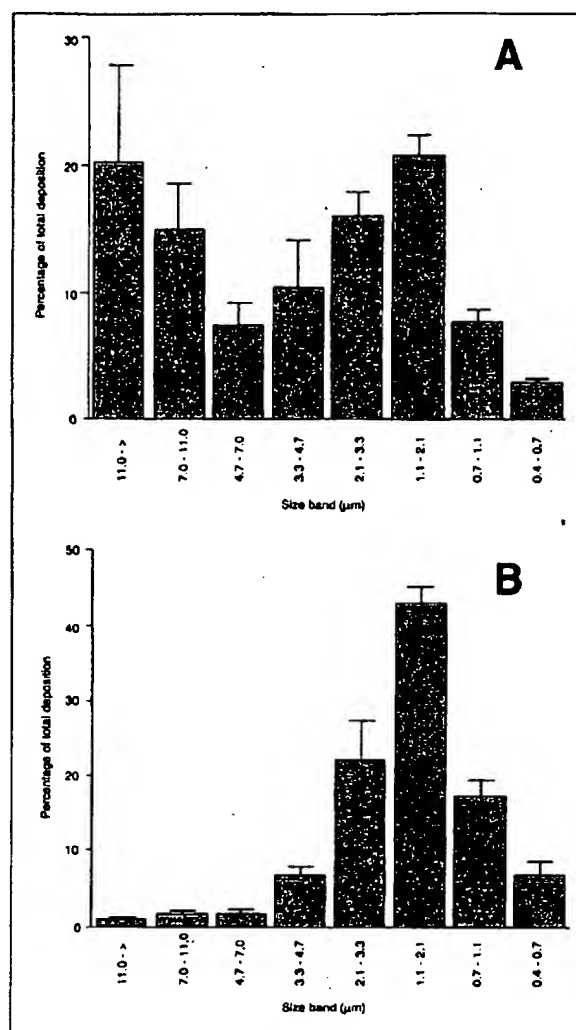


Figure 2 - A : particle size distribution for labelled Teflon microparticles by Andersen method, B : particle size distribution from solution radiolabelled by Andersen method.

possible to reduce this figure despite careful formulation studies, and it was suspected that this consisted of small aggregates of Teflon particles. Electron micrograph measurement of 148 Teflon particles indicated a mass mean aerodynamic diameter of 3.94 μm (geometric standard deviation = 2.31), with a range of 1.11 to 9.0 μm . The micrographs showed well-formed spherical particles with a slight surface roughness.

All aerosols were made up on the day immediately prior to the clinical study due to the short half-life of Tc-99m (6 h). Consequently, it was necessary to make up both the solution and suspension aerosols on several occasions. Hence, confirmation was required that there was no variation between the mass mean aerodynamic diameters of the aerosol formulations for each study day. Unpaired t-tests showed no statistical differences, at the 95% confidence level, between the mass mean aerodynamic diameters of aerosols of the same formulation administered to the different study population

at 1400x

y 5.23 l
volumeAsthma
Queens
study had
lication,
recruited
ears, the
capacity
0.85 to
volume
unction
study day
variation
n forced
y (meaniversity
General
for the
istration

groups (patients and volunteers), but the mass mean aerodynamic diameters of the two formulations were significantly different, both for the batches prepared for the volunteers ($p = 0.0067$) and the patients ($p = 0.0008$).

2. Deposition of aerosols

Tables I and II summarize data describing the total lung deposition, and figures 3a and b show the percentage deposition in each region of interest. The total pulmonary deposition achieved by the solution formulation was significantly greater than that of the suspension formulation in both healthy and asthmatic groups. The solution achieved a total deposition of $61 \pm 11\%$, compared to $17 \pm 5\%$ for healthy subjects ($p = 0.009$), and $59 \pm 17\%$, compared to $10 \pm 4\%$ in asthmatic subjects ($p = 0.004$).

Table I - Total lung deposition of suspension and solution metered dose inhalers in healthy subjects.

ID number	Percentage total lung deposition	
	Suspension metered dose inhaler	Solution metered dose inhaler
1	22.71	72.18
2	14.70	51.26
3	13.90	68.96
4	9.65	48.62
5	15.43	48.37
6	23.15	73.56
7	18.14	71.22
8	11.27	47.24
9	25.43	62.97
10	14.59	55.98
11	22.68	72.10
mean	17.42	61.13
SD	5.32	10.95

Table II - Total lung deposition of suspension and solution metered dose inhalers in patients with asthma.

ID number	Percentage total lung deposition	
	Suspension metered dose inhaler	Solution metered dose inhaler
1	12.71	62.97
2	8.44	54.02
3	16.26	78.67
4	5.57	23.72
5	12.42	77.59
6	10.21	70.19
7	5.27	45.87
8	13.47	65.71
9	4.89	48.54
mean	9.92	58.59
SD	4.41	17.57

The deposition patterns were very similar for the normal and patient groups (figures 3a and b). The suspension metered dose inhaler was characterized by extensive deposition in the head and throat and stomach regions, with uniform deposition in the right and left lungs. The total lung deposition in normals

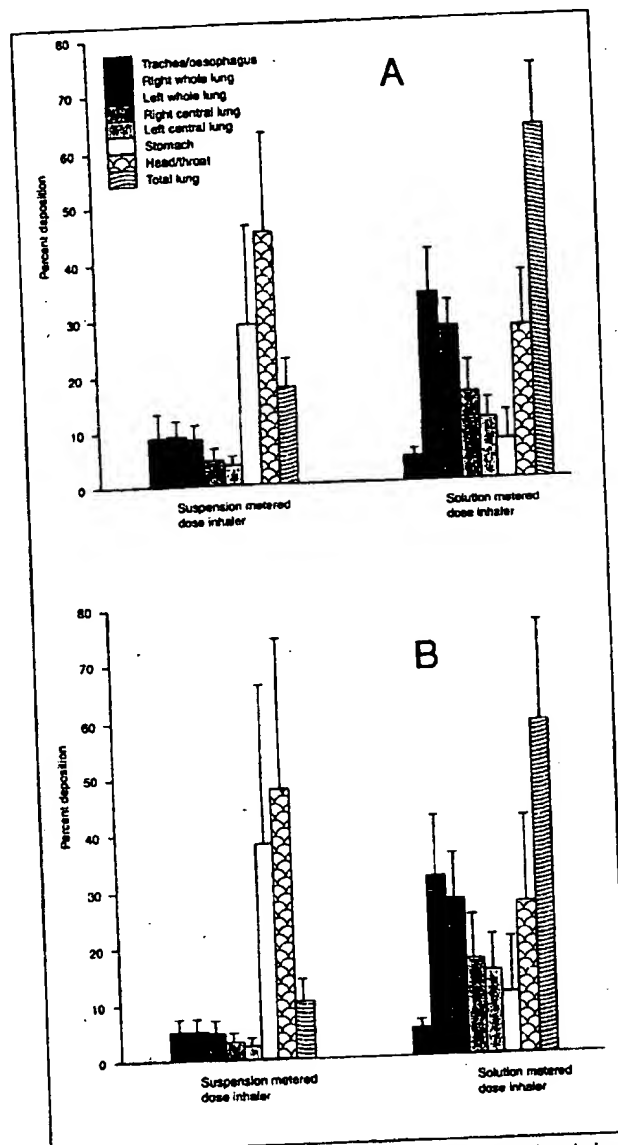


Figure 3 - A : deposition pattern of suspension and solution derived aerosols in normal subjects, B : deposition pattern of suspension and solution derived aerosols in asthmatic subjects.

was 17%, compared to 29% in the stomach and 46% in the head and throat regions. In contrast, the solution metered dose inhaler in normals displayed much higher deposition (61%) in the lung region, with 8 and 27% in the stomach and head/neck regions, respectively. Similar trends were seen in asthmatic patients. When the head/throat and stomach region depositions were added, the suspension-type and solution-type formulations for the healthy subjects and asthmatic subjects showed significant differences. The suspension aerosol had the greater deposition in this combined region ($p = 0.001$ for healthy subjects and 0.0039 for asthmatic subjects, respectively).

III. DISCUSSION

This study compared the performance of a suspension-type

and solution-type metered dose inhaler in healthy and asthmatic subjects. The results show that an approximately threefold increase in lung deposition was achieved with a solution delivered in a metered dose inhaler compared to the suspension delivered in a metered dose inhaler in both healthy and asthmatic subjects.

Labelling of the solution phase aerosol was achieved using technetium-99m-hexakis-(*t*-butylisonitrile) complex, which is more stable than the hexamethylpropyleneamineoxime label used in previous studies by our group. Despite this, the data obtained are comparable with our previous results [5, 6]. The results obtained from the Teflon suspension aerosol are also similar to those reported in both asthmatics and healthy subjects, in which lung deposition ranged from 6 to 18% of the total amount administered [12, 15-18]. It should be noted that the deposition figures quoted here are the percentage of the delivered dose, and do not take into account the impaction loss in the actuator. This normally accounts for 15 to 20% of the total dose, and was a constant factor for all the formulations studied here.

Scintigraphy demonstrates remarkable differences between the deposition patterns of the suspension-type and solution-type metered dose inhalers in both subject groups. This is almost certainly due to the differences in the particle size distribution of the two formulations. The particle size distribution of a metered dose inhaler is an important characteristic of the aerosol, which can influence the particle penetration and deposition in the lung. For example, in a study using the β_2 agonist terbutaline sulphate given as nebulized aerosol from different devices to seven patients with mild asthma, it was found that a significantly better response in forced expiratory volume in 1 s, forced vital capacity and peak expiratory flow was produced when the drug was administered with smaller particle size (mass mean diameter $1.8 \mu\text{m} > 4.6 \mu\text{m} > 10.3 \mu\text{m}$) [19].

The Andersen impactor was designed to classify the particles on the basis of their aerodynamic properties, and so is expected to provide an indication of their likely deposition behaviour *in vivo*. Apart from the significant difference in mean aerodynamic diameter, the Andersen data also reveals differences in the distribution of the aerosols. The solution phase aerosol had 24% of its total mass below $1.1 \mu\text{m}$, while the corresponding figure for the suspension aerosol was only 10%.

In addition to the differences in the particle sizes of the formulations, there is a significant difference in the nature of the inhaled droplets, in that the suspension aerosols consist of solid polymer particles, while the major component of the solution system is the surfactant, which will form an oily semisolid mass once the propellant has evaporated. It is possible that this may create differences in the deposition of the two formulations; in this regard, it should be noted that the effect of particle composition has been poorly investigated, since most published studies are concerned with the deposition of micronized drug powders. This argument assumes, of course, that the propellant has evaporated completely, a point which is still the subject of some discussion.

The Teflon particles studied here are similar to those produced by earlier workers [11] who reported a mass median aerodynamic diameter of $3.2 \mu\text{m}$ (standard deviation = 1.2) compared to $4.12 \mu\text{m}$ (standard deviation = 2.73). Although the Teflon particles produced in this study are slightly larger and have a wider size distribution, the gamma scintigraphy results indicate only small differences in whole lung deposition to the previous studies. A similar finding was reported in a study in which two different sizes of Teflon particles were administered from a pressurized metered dose inhaler to patients with airway obstruction [20]. The Teflon particles had a mass median aerodynamic diameter of $3.2 \mu\text{m}$ (geometric standard deviation = 1.2) or $6.4 \mu\text{m}$ (geometric standard deviation = 1.2). No significant difference was seen between the two formulations, although the smaller particles had the slightly higher deposition.

Although the particle size distribution of the suspension-type and solution-type aerosols explains the differences in deposition pattern between the two systems, it does not explain the lower deposition of the suspension system in the patients with asthma, compared to the normal subjects. It is well documented that respiratory diseases change the airway diameter and geometry of the lungs. In asthma patients, the number of mucus-secreting glands increases, and enlargement of mucus glands results in excessive mucus secretion, accompanied by hypertrophy of airway smooth muscle and inflammation. All these factors contribute to changes in lung morphology that can affect the deposition properties of particles [4]. For example, the narrowing of the airways caused by muscle hypertrophy and inflammation can increase deposition of particles by inertial impaction, and can cause it to occur earlier along the inspiratory path. These changes in lung morphology, and the reduced forced expiratory volume in 1 s of the asthmatic patients, possibly explains the differences in whole lung deposition of the suspension metered dose inhaler compared with the healthy population. This difference is not, however, seen with the solution system in which the same factors are operating. The only reasonable explanation must involve the characteristics of the solution-type aerosol.

The evaporation of aerosol plumes has been the subject of some study. On actuation of a suspension metered dose inhaler, the metered volume of the propellant/drug suspension is released and there is an initial rapid « flashing », during which a fraction of propellant evaporates [21]. The aerosol plume discharged from the metered dose inhaler will contain a distribution of droplet sizes, in which some will contain drug particle while others will consist purely of propellant. The initial size of these droplets depends on the propellant formulation that influences the vapour pressure of the aerosol. The higher the vapour pressure, the greater the « flashing » and thus the smaller the droplets, and the greater the velocity of the aerosol plume [21]. As the propellant droplets travel through the air, further evaporation will occur, but at a slower rate. Particle sizing by holographic microscopy immediately after actuation reveals a plume droplet mass median diameter of $36 \mu\text{m}$ that decreases to $12 \mu\text{m}$ after a distance of 10 cm [22]. Evaporation will continue,

but the limiting factor will be the drug particle size and the number of particles within the initial droplet.

In the case of the solution aerosol, a similar pattern of flashing and distribution of droplet sizes will occur, due to the same propellant formulation and vapour pressure factors. However, the only limiting factor of particle size is the concentration of the solid materials in the propellant. If we compare the typical droplet sizes on actuation (20 to 30 μm) to those of the deposited particles found *in vitro* by the Andersen sampler (1 to 2 μm), it is evident that the vast majority of the propellant (some 99.9% of the total volume) evaporates by the time the droplets impact on the Andersen stage. Thus, we consider that it is justified to interpret the present results as due to the deposition of small particles of radiolabel and surfactant from which the propellant has been lost. *In vivo*, the evaporation of the propellant would be expected to be even more complete due to the higher temperature. For the asthmatic patients, a system such as the solution aerosol may be less influenced by the morphological changes and reduced forced expiratory volume in 1 s than the suspension aerosol, and may explain the scintigraphic deposition results.

*

* *

In this study, we have shown that delivery of a solution phase label from a metered dose inhaler gives much greater whole lung deposition and lower throat/stomach deposition, in both healthy and asthmatic subjects, than a suspended particulate radiolabel. It would appear that formulations of this type have considerable potential for drug delivery to the respiratory system. As a consequence of the greater whole lung deposition, the drug dosage and number of doses could be reduced for the equivalent clinical efficacy, and the fraction of drug swallowed could be minimized, leading to potentially lower incidence of side effects.

REFERENCES

1. Lung and Asthma Information Agency. - Trends in hospital admissions for asthma. - Factsheet 96/2, Dept. of Public Health Services, St. George's Hospital, London, 1996.
2. NEWMAN S.P., AGNEW J.E., PAVIA D. and CLARKE S.W. - Inhaled aerosols: lung deposition and clinical application. - Clin. Phys. Physiol. Meas., 3, 1982.
3. GONDA I. - A semi-empirical model of aerosol deposition in the human respiratory tract for mouth inhalation. - J. Pharm. and Pharmacol., 33, 692-696, 1981.
4. ZANEN P., GO L.T. and LAMMERS J.W.J. - Optimal particle size for β_2 agonist and anticholinergic aerosols in patients with severe airflow obstruction. - Thorax, 51, 977-980, 1996.
5. ASHWORTH H.L., WILSON C.G., SIMS E.E., WOTTON P. and HARDY J.G. - Delivery of a propellant soluble drug from a metered dose inhaler. - Thorax, 46, 245-247, 1991.
6. HARNOR K.J., PERKINS A.C., WASTIE M., WILSON C.G., SIMS E.E., FEELY L.C. and FARR S.J. - Effect of vapour pressure on the deposition pattern from solution phase metered dose inhalers. - Int. J. Pharm., 95, 111-116, 1993.

7. TAYLOR K.M.G. and FARR S.J. - Liposomes for drug delivery to the respiratory tract. - Drug Dev. Ind. Pharm., 19, 123-142, 1993.
8. WALDREP J.C., SCHERER P.W., KEYHANI K. and KNIGHT V. - Cyclosporin A liposome aerosol: particle size and calculated respiratory deposition. - Int. J. Pharm., 97, 205-212, 1993.
9. MAY K.R. - An improved spinning top homogenous spray apparatus. - J. App. Phys., 20, 932-938, 1949.
10. CAMNER P. and PHILIPSON K. - Production of 7 micron monodisperse fluorocarbon resin particles tagged with ^{18}F . - Int. J. App. Rad. Isotopes, 22, 249-353, 1971.
11. NEWMAN S.P., PAVIA D., MOREN F., SHEAHAN N.F. and CLARKE S.W. - Deposition of pressurized aerosols in the human respiratory tract. - Thorax, 36, 52-55, 1981.
12. ZAINUDIN B.M.Z., TOLFREE S.E.J., BIDDISCOMBE M., WHITAKER M., SHORT M.D. and SPIRO S.G. - An alternative to direct labelling of pressurized bronchodilator aerosol. - Int. J. Pharm., 51, 67-71, 1989.
13. ANGELBERGER P., DUDCZAK R., JONES A.G., LISTER-JAMES J., WAGNER-LOFFLER M. and BUCHHEIT O. - Tc-99m-hexakis(1-butylisonitrile)-technetium. I: Optimized synthesis, radiochromatography, biodistribution and preliminary clinical studies. - In: Technetium in Chemistry and Nuclear Medicine, Raven Press, New York, 177-186, 1987.
14. HARDY J.G., JASUJA A.K., FRIER M. and PERKINS A.C. - A small volume spacer for use with a breath-operated pressurized metered dose inhaler. - Int. J. Pharm., 142, 129-133, 1996.
15. KIM C.S., ELDRIDGE M.A. and SACKNER M.A. - Oropharyngeal deposition and delivery aspects of metered-dose inhaler aerosols. - Am. Rev. Resp. Dis., 135, 157-164, 1987.
16. NEWMAN S.P., MOREN F., PAVIA D., LITTLE F. and CLARKE S.W. - Deposition of pressurized suspension aerosols inhaled through extension devices. - Am. Rev. Resp. Dis., 124, 317-320, 1981.
17. MATTHYS H. and KOHLER D. - Pulmonary deposition of aerosols by different mechanical devices. - Respiration, 48, 269-276, 1985.
18. ZAINUDIN B.M.Z., BIDDISCOMBE M., TOLFREE S.E.J., SHORT M. and SPIRO S.G. - Comparison of bronchodilator responses and deposition patterns of salbutamol inhaled from a pressurized metered dose inhaler, as a dry powder and as nebulized solution. - Thorax, 45, 469-473, 1990.
19. CLAY M.M., PAVIA D. and CLARKE S.W. - Effect of aerosol particle size on bronchodilation with nebulized terbutaline in asthmatic subjects. - Thorax, 41, 364-368, 1986.
20. NEWMAN S.P., KILLIP M., PAVIA D., MOREN F. and CLARKE S. - Do particle size and airway obstruction affect the deposition of pressurized inhalation aerosols? - Thorax, 38, 233, 1983.
21. NEWMAN S.P., MOREN F., PAVIA D., LITTLE F., CORRADO O. and CLARKE S.W. - The effects of changes in metered volume and propellant vapour pressure on the deposition of pressurized inhalation aerosols. - Int. J. Pharm., 11, 337-344, 1982.
22. MOREN F. and ANDERSSON J. - Fraction of dose exhaled after administration of pressurized inhalation aerosols. - Int. J. Pharm., 6, 295-300, 1980.

ACKNOWLEDGMENTS

The authors wish to thank Dr. A.H. Short for his advice and help during the preparation and running of the study, and to Ms. E. Blackshaw for assistance with the scintigraphy.

MANUSCRIPT

Received 21 February 1997, accepted for publication 22 May 1997.

Am J Physiol Lung Cell Mol Physiol 277: L1045-L1050, 1999;
1040-0605/99 \$5.00
Vol. 277, Issue 5, L1045-L1050, November 1999

Attenuation of acute lung injury in transgenic mice expressing human transforming growth factor- α

William D. Hardie¹, Daniel R. Prows², George D. Leikauf²,
and Thomas R. Korfhagen¹

¹ Division of Pulmonary Biology, Children's Hospital Medical Center, Cincinnati 45229-3039; and ² Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio 45267-0056

Transforming growth factor- α (TGF- α) is produced in the lung in experimental and human lung diseases; however, its physiological actions after lung injury are not understood. To determine the influence of TGF- α on acute lung injury, transgenic mouse lines expressing differing levels of human TGF- α in distal pulmonary epithelial cells under control of the surfactant protein C gene promoter were generated. TGF- α transgenic and nontransgenic control mice were exposed to polytetrafluoroethylene (PTFE; Teflon) fumes to induce acute lung injury. Length of survival of four separate TGF- α transgenic mouse lines was significantly longer than that of nontransgenic control mice, and survival correlated with the levels of TGF- α expression in the lung. The transgenic line expressing the highest level of TGF- α (line 28) and nontransgenic control mice were then compared at time intervals of 2, 4, and 6 h of PTFE exposure for differences in pulmonary function, lung histology, bronchoalveolar lavage fluid protein and cell differential, and lung homogenate proinflammatory cytokines. Line 28 TGF- α transgenic mice demonstrated reduced histological changes, decreased bronchoalveolar lavage fluid total protein and neutrophils, and delayed alterations in pulmonary function measures of airway obstruction compared with those in nontransgenic control mice. Both line 28 and nontransgenic control mice had similar increases in interleukin-1 β protein levels in lung homogenates. In contrast, interleukin-6 and macrophage inflammatory protein-2 levels were significantly reduced in line 28 transgenic mice compared with those in nontransgenic control mice. In the transgenic mouse model, TGF- α protects against PTFE-induced acute lung injury, at least in part, by attenuating the inflammatory response.

Teflon; ultrafine particulates; macrophage inflammatory protein-2; interleukin-6

This Article

- ▶ [Full Text](#)
- ▶ [Full Text \(PDF\)](#)
- ▶ [Alert me when this article is cited](#)
- ▶ [Alert me if a correction is posted](#)
- ▶ [Citation Map](#)

Services

- ▶ [Email this article to a friend](#)
- ▶ [Similar articles in this journal](#)
- ▶ [Similar articles in PubMed](#)
- ▶ [Alert me to new issues of the journal](#)
- ▶ [Download to citation manager](#)

Citing Articles

- ▶ [Citing Articles via HighWire](#)
- ▶ [Citing Articles via Google Scholar](#)

Google Scholar

- ▶ [Articles by Hardie, W. D.](#)
- ▶ [Articles by Korfhagen, T. R.](#)
- ▶ [Search for Related Content](#)

PubMed

- ▶ [PubMed Citation](#)
- ▶ [Articles by Hardie, W. D.](#)
- ▶ [Articles by Korfhagen, T. R.](#)

This article has been cited by other articles:



Am. J. Physiol: Lung Cellular and Molecular Physiology

[HOME](#)

N. J. Serkova, Z. Van Rheen, M. Tobias, J. E. Pitzer, J. E. Wilkinson, and K. A. Stringer

Utility of magnetic resonance imaging and nuclear magnetic resonance-based metabolomics for quantification of inflammatory lung injury

Am J Physiol Lung Cell Mol Physiol, July 1, 2008; 295(1): L152 - L161.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)



AMERICAN JOURNAL OF Respiratory Cell and Molecular Biology

[HOME](#)

D. R. Prows, A. P. Hafertepen, A. V. Winterberg, W. J. Gibbons Jr., S. C. Wesselkamper, J. B. Singer, A. E. Hill, J. H. Nadeau, and G. D. Leikauf

Reciprocal Congenic Lines of Mice Capture the Aliq1 Effect on Acute Lung Injury Survival Time

Am. J. Respir. Cell Mol. Biol., January 1, 2008; 38(1): 68 - 77.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)



Journal of Applied Physiology

[HOME](#)

J. A. Faress, D. E. Nethery, E. F. O. Kern, R. Eisenberg, F. J. Jacono, C. L. Allen, and J. A. Kern

Bleomycin-induced pulmonary fibrosis is attenuated by a monoclonal antibody targeting HER2

J Appl Physiol, December 1, 2007; 103(6): 2077 - 2083.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)



AMERICAN JOURNAL OF Respiratory Cell and Molecular Biology

[HOME](#)

J. A. Whitsett, C. J. Bachurski, K. C. Barnes, P. A. Bunn Jr., L. M. Case, D. N. Cook, D. Crooks, M. W. Duncan, L. Dwyer-Nield, R. C. Elston, et al.

Functional Genomics of Lung Disease

Am. J. Respir. Cell Mol. Biol., August 1, 2004; 31(2/S1): S1 - S81.

[\[Full Text\]](#) [\[PDF\]](#)



Am. J. Physiol: Lung Cellular and Molecular Physiology

[HOME](#)

J. C. Parker and M. I. Townsley

Evaluation of lung injury in rats and mice

Am J Physiol Lung Cell Mol Physiol, February 1, 2004; 286(2): L231 - L246.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

AMERICAN JOURNAL OF Respiratory Cell and Molecular Biology

[HOME](#)

W. D. Hardie, D. R. Prows, A. Piljan-Gentle, M. R. Dunlavy, S. C. Wesselkamper, G. D. Leikauf, and T. R. Korfhagen



Dose-Related Protection from Nickel-Induced Lung Injury in Transgenic Mice Expressing Human Transforming Growth Factor-alpha

Am. J. Respir. Cell Mol. Biol., April 1, 2002; 26(4): 430 - 437.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)



CHEST

[▶ HOME](#)

G. D. Leikauf, S. A. McDowell, S. C. Wesselkamper, W. D. Hardie, J. E. Leikauf, T. R. Korfhagen, and D. R. Prows

Acute Lung Injury : Functional Genomics and Genetic Susceptibility

Chest, March 1, 2002; 121 (2009): 70S - 75S.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)



AMERICAN JOURNAL OF Respiratory Cell and Molecular Biology

[▶ HOME](#)

D. R. Prows and G. D. Leikauf

Quantitative Trait Analysis of Nickel-Induced Acute Lung Injury in Mice

Am. J. Respir. Cell Mol. Biol., June 1, 2001; 24(6): 740 - 746.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

[HOME](#) [HELP](#) [FEEDBACK](#) [SUBSCRIPTIONS](#) [ARCHIVE](#) [SEARCH](#) [TABLE OF CONTENTS](#)

[Visit Other APS Journals Online](#)